

Commissioner is hereby authorized to charge the required fee of \$110.00 for a one month extension of time to Deposit Account 18-0650.

Prior to examination of the application on the merits, please amend the application as follows:

IN THE SPECIFICATION

Please replace the paragraph starting on page 5, line 18, with the following:

B1 Figure 1 A and 1B- Alignment of CNTF protein sequences. Figure 1A. Human (SEQ ID NO: 1), rat (SEQ ID NO: 2), rabbit (SEQ ID NO: 3), mouse (SEQ ID NO: 4), and chicken (SEQ ID NO: 5) (Leung, et al., 1992, Neuron 8:1045-1053) sequences. Dots indicate residues found in the human sequence. Figure 1B. Modified CNTF molecules (186 [SEQ ID NO: 6], 187 [SEQ ID NO: 7], 188 [SEQ ID NO: 8], 189 [SEQ ID NO: 9], 192 [SEQ ID NO: 10], 218 [SEQ ID NO: 11], 219 [SEQ ID NO: 12], 222 [SEQ ID NO: 13], 223 [SEQ ID NO: 14], and 228 [SEQ ID NO: 15] showing human CNTF amino acid residues (dots) and rat CNTF (residues shown). The name of the purified recombinant protein corresponding to each sequence is shown on the left.

Please replace the paragraph starting on page 8, line 1, with the following:

B2 The above observations led to a directed effort to identify the region on the CNTF molecule responsible for these differences. This method involved the exchange, by genetic engineering methods, of parts of the human CNTF sequence with the corresponding rat CNTF sequence and vice versa. To achieve this, advantage was taken of restriction sites that are common to the two CNTF genes and unique in their corresponding expression vectors. When necessary, such sites were engineered in one or the other of the two genes in areas that encode the same protein sequence. With this approach, expression vectors were obtained for each of the modified proteins shown in Figure 1A and 1B. After expressing and isolating the individual proteins to at least 60% purity, their properties, as compared to those of human and rat CNTF, were determined.

Please replace the paragraph starting on page 16, line 6, with the following:

B3 Plasmid pRPN219 was constructed by first digesting pRPN33 with the restriction enzymes Nhe1 plus Hind3 and gel purifying the 4,081 bp fragment. The second, much smaller fragment which codes for part of the human CNTF gene was subsequently replaced with an 167 bp Nhe1-Hind3 fragment that was obtained by PCR amplification from the rat gene using the primers RAT-III-dniH: 5' ACGGTAAGCT TGGAGGTTCTC 3'; (SEQ ID NO: 18) and RAT-Nhe-I-M: 5' TCTATCTGGC TAGCAAGGAA GATTCGTTCA GACCTGACTG CTCTTACG 3'. (SEQ ID NO: 19).

Please replace the paragraph starting on page 16, line 14, with the following:

B4 Plasmid pRPN228 was constructed in the same manner as pRPN219, except that the 167 bp replacement fragment was amplified using the DNA primers Rat-III-dniH-L-R: 5' AAG GTA CGA TAA GCT TGG AGG TTC TCT TGG AGT CGC TCT GCC TCA GTC AGC TCA CTC CAA CGA TCA GTG 3' (SEQ ID NO: 20) and Rat-Nhe-I: 5' TCT ATC TGG CTA GCA AGG AAG 3' (SEQ ID NO: 21).

Please replace the paragraph starting on page 16, line 20, with the following:

B5 Plasmids pRPN186, pRPN187, pRPN188, pRPN189, pRPN192, pRPN218, and pRPN222 were generated by similar means or by direct exchange of DNA fragments using the unique restriction sites shown in Figures 1A and 1B.

Please replace the paragraph starting on page 20, line 10, with the following:

B6 The expression plasmid pRG632 is a high copy plasmid that encodes ampicillin resistance and the gene for human CNTF-C17A,Q63R Δ C13 (also referred to herein as either AX-1 or AX-13) with a unique Eag I restriction enzyme recognition sequence 3' to the stop codon. This plasmid was used to construct a human CNTF mutation C17A,Q63R, Δ C15 (designated AX-15) by PCR amplification of a 187 bp

Att. Docket No. REG 1
Amendment and Response to Notice
To Comply with Requirements for Patent
Applications Containing Nucleotide Sequence
and/or Amino Acid Sequence Disclosures
and Submission of Formal Drawings

B6
cont.

BseR I-EagI DNA fragment that incorporates the Δ C15 mutation. The 5' primer { Δ C15- 5' (5'-CCAGATAGAGGAGTTAATGATACTCCT-3' [SEQ ID NO: 22])} encodes the BseR I site and the 3' primer Δ C15-3' ((5'-GCGTCGGCCGCGGACCACGCTCATTACCCAGTCTGTGAGAAGAAATG-3'[SEQ ID NO: 23])) encodes the C-terminus of the AX-15 gene ending at Gly185 followed by two stop codons and an Eag I restriction enzyme recognition sequence. This DNA fragment was digested with BseR I and Eag I and ligated into the same sites in pRG632. The resulting plasmid, pRG639, encodes the gene for AX-15 (human CNTF C17A,Q63R, Δ C15). The Δ C15 mutation was then transferred as a 339 bp Hind III-Eag I DNA fragment into the corresponding sites within pRG421, a high copy number expression plasmid encoding the gene for kanamycin resistance and human CNTF C17A,Q63R, Δ C13. The resulting plasmid, pRG643, encodes the gene for AX-15 under transcriptional control of the lacUV5 promoter, and confers kanamycin resistance. The AX-15 gene DNA sequence was confirmed by sequence analysis.

Please replace the paragraph starting on page 25, line 10 with the following:

SEQ ID NO: 16

BT

9	19	29	39	49	59
* *	* *	* *	* *	* *	* *
AFTEHSPLT	PHRRDLASRS	IWLARKIRSD	LTALTESYVK	HQGLNKNINL	DSADGMPVAS
69	79	89	99	109	111
* *	* *	* *	* *	* *	* *
TDRWSELTEA	ERLQENLQAY	RTFHVLLARL	LEDQQVHFTP	TEGDFHQAIH	TLLQVAAFA
129	139	149	159	169	179
* *	* *	* *	* *	* *	* *
YQIEELMILL	EYKIPRNEAD	GMPINVGDGG	LFEKKLWGLK	VLQELSQWTV	RSIHDLRFS
*					
SHQTG					

Please replace the paragraph starting on page 25, line 26, with the following: